The association between serum cytokines and large and small nerve fiber damage and pain in diabetic peripheral neuropathy

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BACKGROUND

Diabetic peripheral neuropathy (DPN) is the most common long-term complication of type 2 diabetes mellitus (DM) and affects approximately half of the patients over the course of disease. Small and large peripheral nerve fibers may be involved in DPN and patients may complain of neuropathic pain (NP). Recent evidence suggests a role of cytokines in DPN and NP.

AIM OF THE STUDY

The paper aimed at exploring whether the serum concentration of cytokines is associated with small and large nerve fiber function and with neuropathic pain (NP).

SUBJECTS AND METHODS

Participants. We recruited 32 subjects (17 men, mean age 63.6 ± 9.3 years, range 47 – 79) who were affected with type 2 DM and fulfilled the following inclusion/exclusion criteria: age ≥ 18 years, no cognitive impairment (Mini Mental State Evaluation score ≥ 25/30), no coexistent neurological disease except for DPN, no severe systemic, infectious or autoimmune diseases, organ failure, hematological diseases or malignancies, no use of analgesic or anti-inflammatory drugs, corticosteroids, or immune modulating therapies.

Clinical and metabolic variables. Body mass index (BMI), waist circumference (WC), therapy for DM (oral hypoglycemic agents, insulin, combined treatment) and measured blood pressure, serum fasting glucose, glycated hemoglobin (HbA1c), creatinine, cholesterol, and triglyceride level, and urinary albumin excretion (UAE) were recorded. All the patients were asked on the presence of pain in the preceding month and pain intensity was measured on a 0 – 10 numerical rating scale (NRS). The presence of probable or definite NP was diagnosed according to the NP grading system.

Electrodiagnostic assessment. All the patients underwent sensory and motor NCS (bandpass filter 10 – 5000 Hz) of the lower limb nerves to explore the function of large nerve fibers. Amplitude of sensory nerve action potential (SNAP) and sensory nerve conduction velocity (SNCV) of the left sural nerve, and amplitude of compound muscle action potential (CMAP), motor nerve conduction velocity (MNCV), and minimal F wave latency of the left peroneal nerve were recorded. SSR to supramaximal median nerve electrical stimulation was recorded (bandpass filter 0.1 – 100 Hz) from the left foot in all the patients.

QST. QST was performed by a trained examiner (F.M.) using a TSA-II NeuroSensory Analyzer (Medoc Ltd., Ramat Yishai, Israel). Thresholds were determined with the Method of Limits. Heat and cold stimuli were delivered through a 30 x 30 mm2 thermode attached to the skin of the dorsal surface of the left foot with a constant pressure. To determine the warm detection threshold (WDT) and the cold detection threshold (CDT), the skin was allowed to adapt to a temperature of 32° C for 5 min and then cooled down or warmed up linearly at a slow rate (1° C/s) until warm and cold sensation was perceived, at which moment the subject stopped the stimulus by pressing a button on a patient response unit. Warm and cold stimulation were repeated four times each and the mean of peak temperatures and vibration was considered threshold. Testing was preceded by detailed instructions to subjects and a demonstration test for each type of stimulus and performed in a designated, quiet room with no distractions. QST evaluation explored the function of small nerve fibers. In particular, WDT and CDT measure C and A-delta nerve fiber loss-of-function, respectively

Blood collection and cytokines assay. Blood samples were obtained in the non-fasting state at the same time of the day (i.e.: 2 pm – 3 pm) in all the patients. Glucose serum concentration at the time of blood sample was checked. After 10 min of rest in the supine position, blood samples were collected from the antecubital vein. Serum and plasma were immediately separated by centrifugation and stored in aliquots at -80°C until analysis. Serum concentration of TNF–α (n. v. < 15.6 pg/ml), IL–2 (n.v. < 31.2 pg/ml), IL–4 (n.v. < 31.2 pg/ml), IL–6 (n. v. < 3.12 pg/ml) and IL–10 (n.v. < 7.8 pg/ml) were assessed in duplicate by an enzyme-linked immunosorbent assay (ELISA) kit (Quantikine, R&D Systems, Minneapolis, USA), according to the manufacturer's protocol.

Table 1: Clinical characteristics of the patients.

	IL-6+ ($n = 14$)	IL-6– ($n = 18$)	IL-10+ ($n = 5$)	IL-10– ($n = 27$)
Age (years)	65.9 ± 9.2	62.4 ± 8.8	68.4 ± 9.4	63.1 ± 8.9
Sex (M/F)	8/6	9/9	4/1	13/14
Disease duration (years)	14.8 ± 8.3	15.2 ± 11.5	18.4 ± 9.5	14.4 ± 10.2
Therapy (oral/insulin/combined)	7/6/1	10/6/2	2/3/0	15/9/3
BMI (kg/m^2)	30.2 ± 6.9	29.3 ± 4.3	25.2 ± 3.2*	30.5 ± 5.5
WC (cm)	108.4 ± 16.2	100.6 ± 10.5	97.6 ± 7.2	105.2 ± 14.3
Hypertension (yes/no)	9/5	11/7	4/1	18/9
Hypercholesterolemia (yes/no)	6/8	9/9	0/5	15/12
Hypertriglyceridemia (yes/no)	3/11	7/11	0/5	10/17
Pain (yes/no)	12/2	14/4	4/1	22/5
Pain severity (0–10 NRS)	2.1 ± 2.3	2.5 ± 2.3	1.9 ± 1.2	2.4 ± 2.4
Serum fasting glucose (mmol/L)	9.0 ± 2.7	9.0 ± 2.8	8.4 ± 2.6	9.3 ± 2.7
HbAlc (mmol/mol)	63 ± 14*	54 ± 12	56 ± 8	58 ± 16
Creatinine (μmol/L)	97.2 ± 53.0	97.4 ± 61.9	106.1 ± 79.6	97.2 ± 53.0
UAE (mg/L)	288 ± 193	210 ± 204	253 ± 235	314 ± 255

BMI: body mass index, WC: waist circumference, NRS: numerical rating scale; HbA1c: glycated hemoglobin, and UAE: urinary albumin excretion from spot urine sample. *$p < 0.05$.

Table 2: Electrodiagnostic measures.

	IL-6+ ($n = 14$)	IL-6– ($n = 18$)	p	IL-10+ ($n = 5$)	IL-10– ($n = 27$)	p
Sural nerve						
SNAP amplitude (μV)	4.4 ± 6.8, 6.6	9.0 ± 6.9, 10.5	0.033	1.6 ± 3.5, 0.8	8.2 ± 7.2, 10.0	0.032
SNCV (m/s)	47.5 ± 9.8, 47.2	45.3 ± 8.5, 43.8	n.s.	49.0 ± 10.9, 47.0	45.8 ± 8.9, 45.3	n.s.
Common peroneal nerve						
CMAP amplitude (mV)	3.2 ± 2.2, 5.3	5.8 ± 3.7, 7.4	0.030	1.0 ± 1.3, 0.5	5.4 ± 3.2, 6.5	0.003
MNCV (m/s)	40.0 ± 6.5, 43.9	42.1 ± 7.0, 44.2	n.s	31.8 ± 5.2, 34.0	42.2 ± 6.1, 44.6	0.009
F-wave minimal latency (ms)	54.7 ± 10.5, 52.0	48.5 ± 6.8, 49.2	n.s.	67.4 ± 13.5, 65.2	49.3 ± 6.5, 49.2	0.042
SSR						
Latency (s)	1.4 ± 0.3, 1.3	1.5 ± 0.2, 1.4	n.s.	1.5 ± 0.1, 1.6	1.5 ± 0.3, 1.4	n.s.
Amplitude (μV)	0.9 ± 0.6, 0.8	1.0 ± 0.7, 1.0	n.s.	1.0 ± 0.3, 0.9	1.0 ± 0.7, 1.0	n.s.

Data are presented as mean ± SD, median. p values are from Mann-Whitney U test. SNAP: sensory nerve action potential, SNCV: sensory nerve conduction velocity, CMAP: compound muscle action potential, MNCV: motor nerve conduction velocity, and SSR: sympathetic skin response.

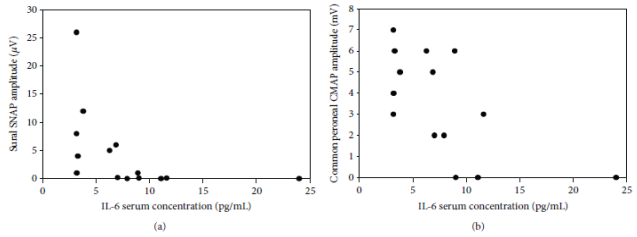

Figure 1: The correlation between the serum concentration of IL-6 and sural nerve sensory nerve action potential (SNAP) amplitude (Spearman's ρ correlation coefficient = −0.085, $p < 0.001$; panel (a)) and common peroneal nerve compound muscle action potential (CMAP) amplitude (Spearman's ρ correlation coefficient = −0.067, $p = 0.009$; panel (b)). For both correlations, Spearman's ρ correlation coefficient turned out to be significant.

Table 3: Quantitative sensory testing measures.

	IL-6+ ($n = 14$)	IL-6– ($n = 18$)	p	IL-10+ ($n = 5$)	IL-10– ($n = 27$)	p
WDT (°C)	42.8 ± 4.9, 42.9	42.1 ± 4.5, 41.4	n.s.	44.0 ± 5.2, 43.7	42.0 ± 4.4, 41.4	n.s.
CDT (°C)	20.9 ± 8.9, 25.9	20.7 ± 9.3, 22.7	n.s.	19.7 ± 8.7, 23.8	21.0 ± 9.2, 24.6	n.s.

Data are presented as mean ± SD, median. p values are from Mann-Whitney U test. WDT: warm detection threshold, CDT: cold detection threshold.

Citokine and NP

No correlation was found between either the positivity or the serum concentration of cytokines and neuropathic pain

CONCLUSIONS

The results of our study show that serum cytokines were abnormally raised in a group of patients with type 2 DM and that this finding was associated with DPN. Raised serum levels of IL–6 and IL–10 correlated with reduced amplitude of sural nerve SNAP and common peroneal nerve CMAP. Raised IL–10 serum level was associated with reduced common peroneal nerve MNCV and delayed minimal F-wave latency. No differences were found in pain characteristics, SSR and QST findings in relation to the presence of raised IL–6 and IL–10. These findings suggest that both cytokines may be related to axonal damage to large but not small nerve fibers and that IL–10 may also be associated with nerve demyelination. These cytokines do not seem to play a direct role in the pathogenesis of NP in our patients. The present data might help understanding the pathogenesis of this complication of type 2 DM and to better target immune-modulating treatments for its prevention and treatment that to date represent an unsolved issue. The cross-sectional nature of the study did not allow understanding the extent to what these abnormalities contribute to nerve damage or represent compensatory or neuroprotective mechanisms. Future studies on larger populations of patients, with a follow-up, and including data from skin and/or nerve biopsies and CSF are needed to overcome the limitations of the present report.